the development following primordia initiation [10]; however, whether environmental factors also impinge upon LR pre-patterning or the events leading up to LR initiation remains unclear. The identification of a link between the output of the LR clock and auxin specifically synthesized at the root's periphery may provide the necessary porthole to begin addressing this question [3]. A role for the root cap in modulating root system architecture via LR outgrowth was previously proposed [11] and the recent data presented by Xuan *et al.* [3] advance this hypothesis. Root cap cells are located at the root–soil interface (Figure 1B,C), and thus, are in an optimal position to serve as sentinels in the coordination of root developmental processes with variable soil conditions.

A fundamental question that persists in regards to LR pre-patterning is how the oscillation in gene expression is translated into a specific developmental response in the xylem pole pericycle cells (Figure 1C). One hypothesis is that the oscillatory signal, reported by DR5 expression in the protoxylem, is transmitted outward to the adjacent xylem pole pericycle cells, thereby 'priming' them for subsequent steps in LR formation [2]. The data presented by Xuan *et al.* [3] show that auxin at the periphery of the root, in the outer root cap cells, is required for LR formation. Integrating these data into the model predicts that two signals, one each from a more interior and more exterior cell type, converge on the xylem pole pericycle cells to establish the distribution pattern of LRs. However, whether the convergence of these signals is required simultaneously or sequentially, the earliest phases of LR formation remains somewhat unclear. Additionally, how cells might be able to distinguish between auxin synthesized specifically in the root cap and auxin synthesized in other cell types or elsewhere in the plant is unknown.

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Heterochromatin: Dark Matter or Variation on a Theme?

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Heterochromatin contributes to the dynamic range of eukaryotic gene expression. In yeast, its ability to suppress transcription is inversely proportional to activator strength. A recent study reveals that Sir silencing proteins enhance the avidity with which nucleosomes assemble, endowing heterochromatin with both repressive and dynamic characteristics.

Heterochromatin, defined as the electron-dense, darkly staining and condensed compartment of the interphase nucleus, has traditionally been considered to be a static and impenetrable nucleoprotein structure. It is the preserve of important structural elements such as centromeres and telomeres (where it plays critical roles

in proper chromosomal segregation and genome stability), but is impoverished in genes, particularly any that are expressed. Over the past two decades, work from a number of laboratories has led to a revision of this 'dark matter' view of heterochromatin. The latest is a paper from Mark Ptashne and colleagues appearing in a recent issue of *Current Biology* [1]. Using as their model the Sir protein-regulated silent chromatin of the budding yeast *Saccharomyces cerevisiae*, these authors demonstrate that while Sir-heterochromatin can extinguish expression of weakly activated genes, it becomes increasingly less effective in repressing transcription as the strength of activation is increased. How might such graduated repression be achieved? The authors demonstrate that one important way Sir proteins silence gene expression is by increasing the avidity with which nucleosomes form over both regulatory and coding regions of genes. Nucleosomes are the fundamental repeating subunit of chromatin, with most of the eukaryotic genome assembled into these ~200 bp bead-like, histone-containing structures. The ability of stably bound nucleosomes to inhibit transcription has been documented in cell-free systems [2], but it is less clear under what circumstances or contexts they do so *in vivo*.

The fact that a potent gene-specific activator can override the repressive effects of yeast heterochromatin is not new. This was originally demonstrated by studies using either a telomere-linked *URA3* transgene or a heat shock gene flanked by short DNA sequences termed silencers. In both settings, Sir2/3/4 proteins are recruited, and they in turn bind nucleosomes that are assembled into silent chromatin. In the case of *URA3-Tel*, the activator protein Ppr1 could instigate activation only when overexpressed and only during a specific window of the cell cycle (G2–M) [3]. In the case of the silencer-flanked heat shock gene, the Hsf1 activator was shown to occupy its normal binding site even in the basal state, circumstances in which the Sir2/3/4 complex repressed transcription up to 100-fold. Following a heat stimulus, Hsf1 was able to readily overcome Sir-repression, driving a rapid, several hundred-fold increase in expression [4,5]. These earlier observations can now be understood in terms of Sir heterochromatin being a dynamic structure whose repressive activities compete with activators that co-habitat the same domains. The stronger the activator (i.e., the more efficient its recruitment of coactivators, chromatin remodeling enzymes, and the general transcriptional machinery), the more likely that RNA polymerase (Pol II) recruitment to the promoter, Pol II escape from the promoter, and productive Pol II elongation over the gene coding region will occur. Each of these steps can be potentially regulated by Sir heterochromatin [5–7].

Similarly, it is now evident that Polycomb Repressive Complex 2 (PRC2)-mediated repression, a widespread mechanism signified by the presence of the histone H3 K27me3 covalent modification, reduces occupancy of paused Pol II within promoter-proximal regions yet does not obviate it [8]. At such genes, Polycomb appears to be permissive to some Pol II recruitment, promoter escape and early elongation, yet prohibits productive elongation. Polycomb-repressed chromatin has classically been referred to as 'facultative' (regulated) heterochromatin [9] and is typically associated with developmental and tissue-specific genes whose expression is tightly regulated; it also underlies mammalian X chromosome inactivation. Interestingly, this form of heterochromatin, like its yeast counterpart, can be overcome by action of powerful transcriptional activators that bind *cis*-acting enhancer elements [10].

A distinct form of heterochromatin, sometimes referred to as 'constitutive heterochromatin', is found at telomeres, centromeres and pericentric (centromere-flanking) regions in many metazoans, including mammals, insects, plants and fungi. It is also formed at repetitive satellite DNA sequences and transposons, and remains condensed throughout the cell cycle. Its assembly is mediated by heterochromatin protein 1 (HP1) and is linked to two covalent histone marks, H3 K9me3 and H4 K20me3, as well as 5-methyl cytosine DNA methylation. Despite its repressive nature, constitutive heterochromatin is dynamic, as HP1 is constantly being recruited to ensure maintenance of the silenced state [11]. In this regard, HP1-heterochromatin bears a strong resemblance to Sir-heterochromatin that likewise is accessible to regulatory factors [5,6] and requires the continuous recruitment of Sir proteins for its maintenance and propagation [12].

The small interfering RNA (siRNA)-dependent heterochromatin found in the fission yeast *Schizosaccharomyces pombe*, is also likely to be dynamic. This organism evolved an entirely distinct mechanism for silencing than that used in the distantly related *S. cerevisiae.* Instead of *cis*-acting silencer elements, *S. pombe* expresses long non-coding RNAs (lncRNAs) at the regions to be silenced — outer centromeric repeats, telomeres and silent mating loci — that serve to tether an siRNA-containing complex, RITS, that binds the lncRNAs through base-pairing interactions. RITS in turn recruits, directly or indirectly, factors that trigger formation of heterochromatin, including an enzyme that trimethylates histone H3 K9 and orthologues of HP1 and the Sir2 silencing protein [13]. (See Figure 1 for a summary of this and the other heterochromatic mechanisms.)

What then do these forms of heterochromatin have in common, the property that permits them to be highly effective repressors of basal transcription, yet exhibit sufficient plasticity to allow strong activators to overcome silencing? The common thread is the presence of histone deacetylase complexes (HDACs), enzymes that remove acetate groups from lysine residues. In the case of Sir-mediated heterochromatin, the lysine deacetylase is Sir2, founding member of the Sirtuin family of NAD^+-dependent HDACs. Sir2 exhibits especially strong reactivity towards acetylated lysine residue H4 K16, a key lysine whose unacetylated state is critical to the folding of the 'beads-on-a-string' chromatin filament into a compact, 30 nm-like fiber [14] (Figure 1). Importantly, histone methylation, unlike acetylation, does not directly affect nucleosome structure. Instead, this modification creates novel surfaces recognized by proteins that contribute to chromatin remodeling and transcriptional regulation, including

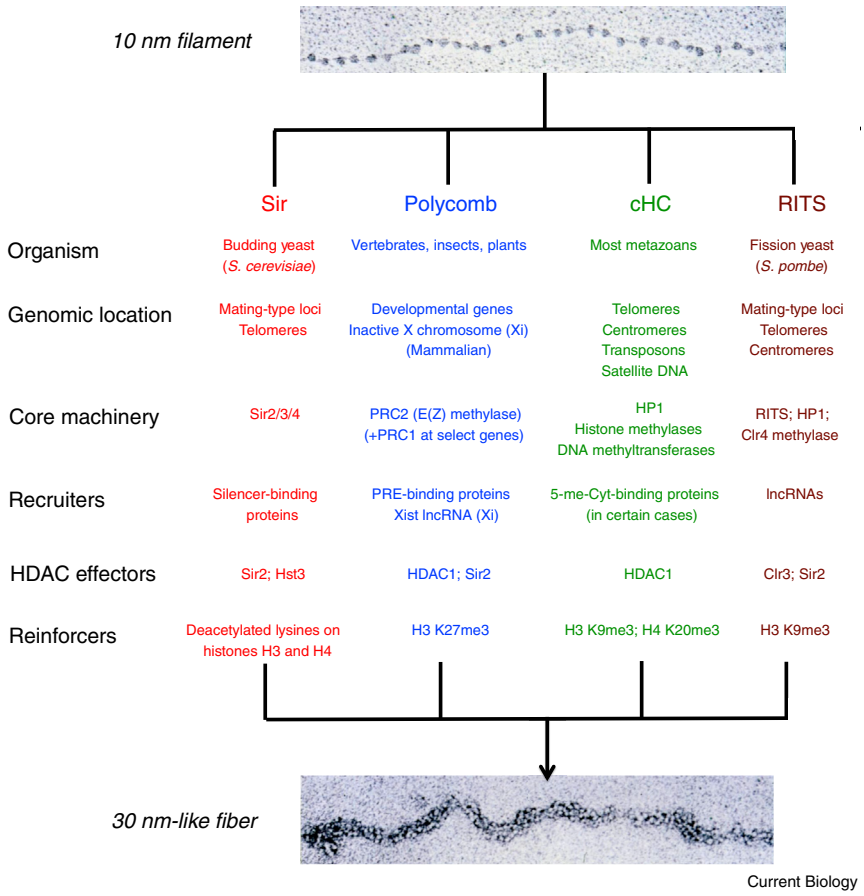

Figure 1. Heterochromatin: variations on a theme.
Schematic illustrates (top to bottom) the common starting point for heterochromatin assembly, the beads-on-a-string 10 nm filament; four evolutionarily distinct mechanisms of heterochromatin formation; and the product of heterochromatinization, the condensed 30 nm-like fiber. Not shown are components of the complexes underpinning each mechanism; these bind to individual nucleosomes within the chromatin fiber. 'Reinforcers' are covalent histone modifications to which one or more constituents of the core machinery bind. cHC, constitutive heterochromatin; RITS, RNA-induced initiation of transcriptional gene silencing; PRE, Polycomb response element; 5-me-Cyt, 5-methyl cytosine.

the HP1, RITS and PRC2 complexes themselves. Thus, H3 K9me3 and H3 K27me3 marks stabilize the interactions between the heterochromatic machinery and the underlying nucleosomes.

Wang *et al.* [1] through use of a powerful, quantitative micrococcal nuclease digestion assay developed in the Ptashne lab, show that Sir heterochromatin, and by implication other forms of heterochromatin, exert at least part of their repressive effects in the most fundamental way — by increasing the capacity of nucleosomes to form on the underlying DNA. They observed that nucleosome occupancy of the *HMR* locus, which harbors the cell identity gene pair *a1/a2*, increased from 30–40% in the absence of Sirs (i.e., in a *sir*$^-$ mutant), to 50–60% in their presence [1]. Importantly, the authors observed this effect under circumstances in which the genes located at *HMR* were transcriptionally inactive irrespective of the presence of Sirs. Therefore, absence of transcription *per se* cannot explain the increased nucleosomal stability seen in the *SIR*$^+$ cells, and indicates that such increased avidity is a cause, and not a consequence, of transcriptional silencing. Consistent with this, Sirs recruited by flanking *HMR* silencers strongly suppress the nucleosomal disassembly that normally accompanies transcriptional activation of a heat-inducible gene, dampening its expression [15]. Also of relevance, a recent study of Dodson and Rine [16] demonstrated that the extremely low transcription emanating from the yeast *HMR* locus is a consequence of rare derepression — occurring in only 1 in 1,000 cells. This study, in combination with that of Wang *et al.*, points to the exceptional stability of the *HMR* heterochromatic domain.

In sum, heterochromatin — from yeast to human — is not a static, inert structure but rather a dynamic form of chromatin, whose constituent proteins are continuously exchanging. It has evolved in different eukaryotes using unique constituents and mechanisms for assembly, yet its function is largely the same: to stabilize the genome and increase the fidelity with which it is transmitted, and to suppress unwanted basal transcription while allowing activated gene expression in a context- and cell type-specific manner.

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Evolution: A Genomic Guide to Bird Population History

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How species responded to the climatic oscillations during the past few million years is debated. A new study analyzing the genomes of 38 bird species finds variable patterns of population growth and declines that broadly correlate with global environmental change.

In a recent interview, Richard C. Lewontin, a central thinker in the field of evolutionary biology, succinctly noted that the "admission of necessary ignorance of historically remote things is the first rule of intellectual honesty in evolution" [1]. Possibly with reluctance, many biologists would admit to some level of ignorance in terms of our understanding of how different species responded to the extreme variation in climate over the past few million years. This is important, given that many of the species studied today presumably went through major shifts in their habitat and ecology during these times. Much of our understanding of the historical changes in the distribution and abundance of species have been gleaned from diverse sources, including fossils and pollen depositions [2]. Now, a recent paper in *Current Biology* by Nadachowska-Brzyska *et al.* [3] brings genomic data to bear on the question of how different species may have responded to these tumultuous times.

Extracting historical information from the genomes of extant individuals is a challenging affair. This is primarily because most methods used today provide only a snapshot of the recent past. For example, nucleotide variation in a species' DNA can be used to indirectly estimate historical population sizes, whereby low levels of genetic variation suggest that its recent ancestral population was small. However, these statistics tells us little about what may have happened earlier — was this ancestral population preceded by a much larger group of individuals?

Recently, researchers have been able to work around these limitations by applying sophisticated modeling approaches to genomic data. These methods rely on the premise that different genomic regions within an individual's DNA may provide semi-independent pieces of information from distinct historical time periods. One such analytical method that has become popular, pairwise sequentially Markovian coalescent modeling (PSMC), generates inferences about historical effective population sizes by using genome-wide sequencing data from a single individual [4].

Effective population size is a central metric in the field of population genetics: it considers only those individuals in a population that pass on genes to the next generation and, depending on the demographics, can be quite different from a population's census size [3]. The PSMC framework was originally developed to quantify historical variation in effective population sizes in humans, although it has also been used to study patterns in pigs [5], horses [6] and other taxa [7]. In their study, Nadachowska-Brzyska *et al.* [3] mine 38 of the 48 recently published bird genomes [8] to quantify how population sizes of avian species changed globally over the past few million years. Avian systems, in particular, have a rich legacy of research into the patterns of diversity and historical biogeography [9,10].

The PSMC method employed by Nadachowska-Brzyska *et al.* [3] takes advantage of variation in the extent of heterozygosity (a simple measure of genetic variation) throughout the genome of diploid individuals. At this scale, heterozygosity can be influenced by a number of factors, but in this case it is assumed to be primarily affected by the effective population size. For